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An Investigation of the Vitamin-A Content of Certain Fish Oils using the Colour Test

By H. Ritchie Chipman

An article in Canadian Chemistry and Metallurgy, (1927, XI. 235) by Horace N. Brocklesby of the Fisheries Experimental Station, (Pacific) compares the vitamin-A content of dogfish liver oil with cod oil, employing feeding experiments on rats. The Fisheries Experimental Station, (Atlantic) has recently been interested in the colour test for vitamin-A using a solution of antimony trichloride in chloroform, and it was decided to apply this test to some dogfish liver oil and compare it with some samples of cod liver oil.

The method of making the test consist in dissolving a few drops of the oil in chloroform, taking a small part of the resulting solution and adding a small amount of a 30% solution of antimony trichloride in chloroform, the formation of a blue coloration denoting the presence of vitamin-A. The authors of the test tried it against feeding experiments and found that the depth of the colour was proportional to the vitamin-A content. The authors standardized the colour by means of a Lovibond tintometer. A tintometer was not available at the Experimental Station, and attempts were made to find some solution which would have a sufficiently good colour for matching and which could easily be made up to give the correct colour. Various solutions of methylene blue were tried but it was found that they were only suitable for a certain part of the colour range.

It was suggested by Dr. Leim, of this Station, that an indicator could be found which would give the correct colour range and which could be made up at any time. An glance at an indicator colour chart showed that Thymol Blue in a pH of 8.6 to 10.0 gave a colour range very suitable for matching the colours produced during the vitamin test. The proper buffer solutions were made up and a series of colour tubes prepared.

Having at hand a suitable means of comparing the colour produced it was decided to apply the test to some fish oils which happened to be on hand, and to prepare oil from the livers of the dogfish, haddock, and hake for a comparison with cod liver oil.

In the preparation of the liver oils, the livers were removed immediately after the fish was caught, washed several times, and then cut up into small pieces. The liver was then placed in a large beaker and steam passed in for an hour. During this process the liver became darker, broke up somewhat, and the oil appeared on the top of the water which condensed from the steam. After steaming the beaker was allowed to cool and the solid pieces of liver strained off by a wire screen. The liquid was allowed to settle and the aqueous layer drawn off by means of a separatory funnel. The oily layer was then filtered through charcoal after being allowed to stand for some time. The oil from the livers of the dogfish and haddock came through as a clear oil but that from the hake was rather cloudy.

The colour test was then applied to these liver oils as well as to some other oils which happened to be on hand. In applying this test some slight departures were made from the original procedure and a method was finally adopted which allowed consistent results.

In the method adopted, ten drops of the oil were placed in a test tube and 5 cc. of chloroform were added. The tube was well shaken and 2 cc. of a 30% solution of antimony trichloride in chloroform were then added. The blue colour formed immediately and the depth of colour was measured by comparing immediately with the standard tubes containing the thymol blue. The colour formed in the test does not last long and the colour had to be compared immediately or it would be lost. However the colour lasted long enough to get a good comparison.

To ensure correct measurements, tubes were marked so as to show the liquid level at 5 cc. + 10 drops, and again at an additional 2 cc. This made the use of pipettes or other measuring devices quite unnecessary.

The colour range given by the thymol blue standards was a good match for the colours except when the amount of vitamin A was large. After a certain depth of colour had been reached the resulting blue was of a shade which could not be properly matched against the thymol blue. But this only took place in a few cases and denoted a large amount of the vitamin.

The following results were obtained upon a comparison of the fish oils which were available. The figures given denote the standard colour tube. These standards were numbered arbitrarily from 1 to 10.

Cod liver oils	
Nadruco, Norwegian, best,	1.5
National Fish Co.	4.5
Bishops, Nova Scotia Oil	8.0
Munn's Newfoundland refined,	5.0
Fasterfat cod oil	5.0
Munn's Cod oil	6.5
Dogfish Liver oil	10
Hake liver oil	5.0
Haddock liver oil	0
Herring oil	0
Salmon	0

These results show an interesting diversion among the cod liver oils. The Nadruco oil was supplied by the National Drug Co. at Halifax as their best available cod liver oil. It will be seen that it cannot compare with any of the others in its vitamin A content. The effect of refining shows in the figures obtained in the case of Munn's oils, where the refined oil is lower in vitamin A content than the unrefined cod oil. Bishop's oil, which is made by a firm in Liverpool, N.S. shows a vitamin A content even better than the unrefined Newfoundland oil.

The dogfish liver oil seemed to have a vitamin A content much greater than any of the others. This seems to agree with results of feeding experiments carried out by Brocklesby, and by Holmes and Pigott, (J.I.E.C., 1925, XVII, 210). Brocklesby points out in his paper that his work has been done on oil from the California dogfish, Squalus Sucklii, while the work of Holmes and Pigott has been done on oil from the Atlantic dogfish, Squallus Acanthias. Both species are quite similar, and the feeding experiments of these investigators show the dogfish liver oil to be at least as good as cod liver oil, if not better.

The hake liver oil seems to compare very favourably with the cod and dogfish liver oils. The result from the haddock liver oil was rather surprising as it would be thought that it would be almost as good as cod liver oil. The free use of haddock livers in the preparation of "cod liver oil" may account for the wide variations in medicinal oils.

The herring and salmon oils showed no vitamin A content whatever. These oils were not liver oils, but were obtained by extraction from the flesh of the fish.